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Synthesis and [2 + 2]-photodimerisation of monothiomaleimide functionalised linear and brush-like polymers

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[2 + 2]-Photodimerisation of monothiomaleimides has been demonstrated on functionalised linear and brush-like polymers. In water/acetonitrile (95 : 5) mixtures the rate of reaction is accelerated significantly by irradiation of the thiomaleimide end group ($\lambda_{\text{max}} = 350 \text{ nm}$) with UV light, reaching full conversion within 10 minutes.

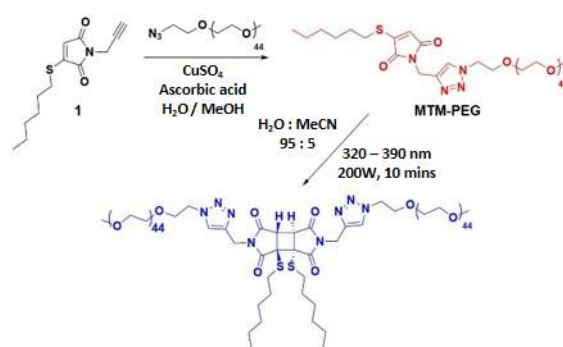
[2 + 2]-Photocycloadditions are useful reactions in organic and polymer synthesis as well as materials fabrication.¹ They are formally forbidden under thermal conditions offering orthogonality with respect to thermal cycloadditions and other thermally triggered reactions, whilst providing regio- and stereochemical control over the formation of challenging cyclobutane scaffolds.² In polymer and materials science, the rational design of chromophores has significantly improved reaction efficiency and led to the development of tuneable and reversible [2 + 2]-photoligation reactions that can even occur in the visible region with appropriately conjugated alkenes or in the presence of triplet sensitisers.^{3–5}

Maleimide's exhibit diverse reactivity owing to the relatively low energy $\pi^*_{\text{C}=\text{C}}$ orbital within the cyclic imide structure which provides an efficient handle for post polymerisation modification, bioconjugation, polymer crosslinking and surface modification.^{6–9} Maleimides are also photoactive and can undergo inter and intramolecular [2 + 2]-photocycloadditions in both batch and continuous flow reactions.^{10–12} Under batch conditions, the [2 + 2]-photocycloaddition of maleimide is relatively sluggish, requiring long irradiation times with high energy UV-light (typically 270 – 320 nm) which can compromise structural integrity, particularly when employing such modifications to functionalised biomolecules.¹³ Triplet sensitisers such as thioxanthone undergo excitation at longer wavelengths and can enable [2 + 2]-photocycloadditions to be

performed in the visible region.^{14,15} Two-photon processes have also been developed in which near-IR light is capable of promoting reactions.¹⁶

Recently, mono- and disubstituted thiomaleimides (MTM, DTM) have been developed as efficient and highly specific reagents for bioconjugation and polymer ligation.^{17–24} The thiomaleimides are synthesised by reaction of mono and dibromomaleimide precursors with a desired thiol compound which can be followed colourimetrically since substitution of the bromine group(s) by the thiols proceeds with the emergence of a strong yellow solution.²⁵ The colour change represents a red shift in the λ_{max} of the maleimides from <280 nm for unsubstituted maleimide to 339 nm for monothiomaleimides and 393 nm for dithiomaleimides respectively. In light of this, [2 + 2]-photocycloaddition of thiomaleimides were investigated by Baker and co-workers, who demonstrated that highly efficient (5 minutes irradiation), stereo- and regiospecific (*exo*, head-to-head) photodimerisation and cycloaddition reactions took place in 95 : 5 H₂O/acetonitrile mixtures, under irradiation at 365 nm at concentrations as low as 72 μM .¹⁷ These conditions were later shown to be suitable for bioconjugation, whereby [2 + 2]-photodimerisation was shown to enable efficient disulfide rebridging of a therapeutic peptide and antibody fragments.²⁶ The reactivity and ability to tune the absorption wavelength of thiomaleimides is an attractive property for applications in polymer and materials science.

Scheme 1. Proposed route to the dimerisation of **MTM-PEG** via [2+2] photocycloaddition.



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In order to investigate [2 + 2]-photodimerisation of MTM-groups attached to polymer substrates (Scheme 1), *N*-propargyl-3-monothiohexylmaleimide (**1**) was synthesised in a two-step reaction (Scheme S1). The second step of this synthesis involves addition of hexanethiol to the *N*-propargyl-3-bromomaleimide in a 1 : 1 ratio at room temperature which was complete in 5 mins according to the literature.¹⁷ However, the yield of **1** was limited to 40 % in our hands. This is due to addition of a second molecule of hexanethiol resulting in formation of the dithiosuccinimide in 30 % yield (note; nucleophilic thiol-yne addition between alkyne and thiol could also occur as unwanted side reactions). To suppress these proposed side reactions, the reaction temperature was reduced to -78 °C which increased the yield of **1** to 75%. The structure of **1** was confirmed by ¹H and ¹³C NMR according to the shift of the olefin signal from 6.90 ppm to 6.02 ppm and 131.8 ppm to 152.2 ppm respectively (Fig. S1). The relative integration of the olefin, alkyne, and methyl protons from hexanethiol at 6.02 ppm, 2.14 ppm, and 0.83 ppm respectively was 1 : 1 : 3 indicating that the thio-Michael addition and thiol-yne side reactions had been suppressed at the lower reaction temperature. UV-vis spectroscopy is a useful method to follow manipulations of the maleimide group. Maleimide has a λ_{max} ≈ 273 nm which is red shifted upon substitution to form monobromo- and dibromomaleimide respectively. Further substitution of the C-Br bonds by addition-elimination resulting in monothiol- and dithiomaleimides results in a larger red shifts to λ_{max} = 339 nm and 393 nm respectively and an increase in the extinction coefficient. Here, **1** gave absorption with λ_{max} = 350 nm (Fig. S2).

The retention of the alkyne group, as required for polymer functionalisation, was further supported by FTIR, which showed a peak at 3270 cm⁻¹ (Fig. S3). This is a useful handle to follow the copper catalysed azide-alkyne click (CuAAC) reaction with the complementary azido-functional polymers. Commercially available α -methoxy- ω -azido poly(ethylene glycol) (**N₃-PEG-OMe**) was selected as the azido-functionalised polymer. The CuAAC reaction between **N₃-PEG-OMe** and **1** was performed the CuSO₄ / ascorbic acid catalyst system in an aqueous alcohol solvent.²⁷ The CuAAC reaction was successful, with formation of *N*-propargyl-3-monothiohexylmaleimide functionalised PEG (**MTM-PEG**) initially confirmed using FTIR by the absence of the alkyne and azide functional groups of the starting reagents at 3270 cm⁻¹ and 2096 cm⁻¹ respectively (Fig. S3). Furthermore, the product also exhibited a peak corresponding the imide group at 1710 cm⁻¹. In ¹H NMR, the presence of a triazole ring proton at 8.0 ppm, integrating at a 1 : 1 ratio with the thiomaleimide proton signal at 6.35 ppm indicated that end-group modification was 100% (Fig. 1A).

Size exclusion chromatography (SEC) unexpectedly revealed a bimodal molecular weight distribution (M_n = 1700 g.mol⁻¹; \bar{D} = 3.52). Under the chosen reaction (and purification) conditions, the CuAAC reaction and subsequent aqueous dialysis mixture were exposed to natural light for > 24 hours. It was hypothesised that the [2 + 2]-photodimerisation could be taking place slowly in the natural laboratory light, resulting in the formation of a mixture of the desired **MTM-PEG** and the dimerised product,

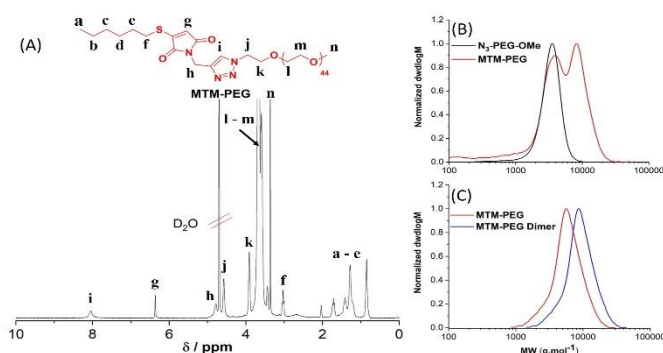


Fig 1. ¹H NMR of **MTM-PEG** in D₂O solvent (A); SEC traces of **MTM-PEG** synthesised in the presence of natural light showing a bimodal distribution (B) and with strict exclusion of natural light (M_n = 4100 g.mol⁻¹, \bar{D} = 1.2, red) along with its dimer (M_n = 7900 g.mol⁻¹, \bar{D} = 1.2, blue) (C).

manifest as the bimodal distribution of the SEC trace (Fig 1B). The CuAAC reaction and subsequent purification were repeated with strict exclusion of natural light. Pleasingly, SEC analysis subsequently revealed a monomodal molecular weight distribution (M_n = 4100 g.mol⁻¹; \bar{D} = 1.2, Fig. 1C, red).

The [2 + 2]-photodimerisation of **MTM-PEG** was initially investigated in a water/acetonitrile mixture (95 : 5) at a concentration of 20 mg .ml⁻¹ using a commercially available UV nail gel curing lamp containing four 9 W bulbs (λ_{max} ≈ 365 nm) for irradiation. Dimerisation was confirmed by ¹H NMR based on disappearance of the olefinic proton at 6.35 ppm (Fig S4). The appearance of peaks corresponding to the cyclobutane motif (H_j, Fig S5), and the change in chemical shift and environment for the methylene protons adjacent to the thio-group (H_{c,c'}, Fig S5) is consistent with the experimental results reported in the literature, and with FMO theory to deduce *exo*-head-to-head photodimerisation products of small molecule MTM analogues.¹⁷ The number average molecular weight increased from M_n = 4100 g.mol⁻¹ to M_n = 7900 g.mol⁻¹ and a monomodal molecular weight distribution was retained (\bar{D} = 1.2, Fig. 1C, blue). MALDI-ToF-MS analysis, in reflectron mode, gave poor resolution but qualitatively supported dimerisation with a shift in the *m/z* distribution (Fig. S6).

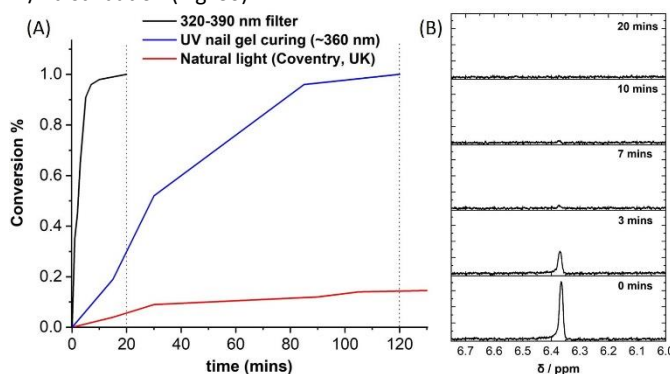


Fig 2. Kinetics of the [2 + 2]-photodimerisation of **MTM-PEG** in D₂O/CD₃CN solvent (B); y-axis represents the conversion to dimer and x-axis represents the time of irradiation (A); representative data collected using ¹H NMR following the signal of the olefinic proton of **MTM-PEG** using Omnicure s2000, 200 W, λ = 320-390 nm in D₂O/CD₃CN solvent (B).

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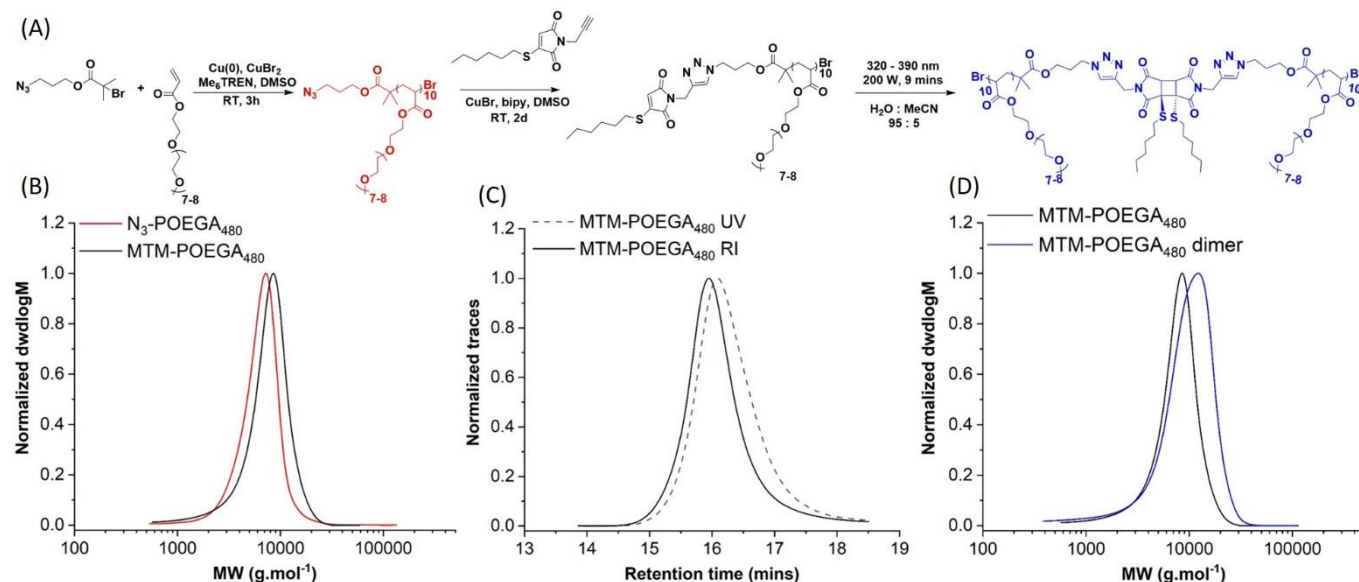


Fig 3. General scheme for the synthesis and [2 + 2]-photocycloaddition of **MTM-POEGA₄₈₀** (A); SEC traces for the synthesis of **N₃-POEGA₄₈₀** ($M_n = 5400 \text{ g.mol}^{-1}$ and $\bar{D} = 1.26$, red) and **MTM-POEGA₄₈₀** ($M_n = 6000 \text{ g.mol}^{-1}$ and $\bar{D} = 1.36$, black) (B); coinciding RI and UV ($\lambda = 320 \text{ nm}$) for **MTM-POEGA₄₈₀** (C); dimerisation of **MTM-POEGA₄₈₀** upon irradiation (200 W, $\lambda = 320\text{--}390 \text{ nm}$; $M_n = 6400 \text{ g.mol}^{-1}$; $\bar{D} = 1.67$, blue) (D).

In order to investigate the kinetics of the [2 + 2]-photodimerisation in presence of different light sources, NMR experiments were performed and disappearance of the olefinic proton at 6.35 ppm was monitored (Fig 2B). Three different light sources were investigated including the broad polychromatic UV curing lamp (4 x 9 W; $\lambda_{\text{max}} \approx 365 \text{ nm}$), a more focused mercury lamp with a bandpass filter (Omnicure s2000, 200 W, $\lambda = 320\text{--}390 \text{ nm}$), and the natural light of the lab (Fig. 2A). The UV curing lamp reached full conversion after 120 minutes, while using the more powerful and filtered mercury lamp (200 W, $\lambda = 320\text{--}390 \text{ nm}$) increased the rate of reaction leading to completion within 10 minutes. The emergence of the cyclobutane protons and shift in the methylene protons adjacent to the thio-group coincided with the disappearance of the olefinic proton (Fig S7). In the presence of natural light dimerisation occurred at a significantly lower rate. For example, after 20 minutes of exposure, conversion reached < 10 % whereas the UV curing lamp reached $\approx 30\%$ conversion and higher power, filtered light afforded by the mercury lamp achieved full conversion. Finally, to support the outcome of the ^1H NMR experiments, the [2 + 2]-photodimerisation of **MTM-PEG** using the mercury lamp (200 W, $\lambda = 320\text{--}390 \text{ nm}$) as an irradiation source was followed by UV-vis spectroscopy (Fig S8). Pleasingly, the absorbance attributed to the MTM end-group ($\approx 350 \text{ nm}$) decreased significantly within 10 minutes, showing no further change with continued irradiation suggesting that the reaction was complete within 10 minutes which corroborates the outcomes of the NMR experiments.

Having demonstrated the dimerisation of **MTM-PEG**, the compatibility of the [2 + 2]-photodimerisation with brush-like polymers synthesised by Cu(0)-mediated reversible deactivation radical polymerisation (RDRP) was investigated (Fig 3A). A previously reported azido-functionalised initiator²⁷ was synthesised (Fig. S9, S10) and oligo(ethylene glycol) acrylate ($M_n = 480 \text{ g.mol}^{-1}$; OEGA₄₈₀) was selected as the monomer to ensure the resulting polymer ($\text{DP}_{n,\text{th}} = 10$) would be soluble in the same water / acetonitrile (95 : 5) solvent system employed for the [2 + 2]-photodimerisation reaction of the **MTM-PEG**. The polymerisation of OEGA₄₈₀ was performed in DMSO using Cu(0)-wire (5 cm) and $[\text{I}] : [\text{OEGA}] : [\text{CuBr}_2] : [\text{Me}_6\text{Tren}] = [1] : [10] : [0.05] : [0.18]$, in line with previous reports (Fig. 3A).²⁸ Conversion reached >95% within 3 hours (Fig. S11) and SEC indicated that the polymerisation was well controlled furnishing **N₃-POEGA₄₈₀** with $M_n = 5400 \text{ g.mol}^{-1}$ and $\bar{D} = 1.26$ (Fig. 3B, red). The **N₃-POEGA₄₈₀** was converted into **MTM-POEGA₄₈₀** upon CuAAC reaction with **1** using Cu(I)Br and bipyridine as the catalyst in this case.²⁹ Evidence of successful CuAAC reaction taking place was provided by SEC which revealed coinciding RI ($M_n = 6000 \text{ g.mol}^{-1}$ and $\bar{D} = 1.36$) and UV ($\lambda = 320 \text{ nm}$) traces for the functionalised polymer (Fig. 3C), which was absent in **N₃-POEGA₄₈₀** (Fig S12). Although ^1H NMR supported successful click reaction, the degree of functionalisation was calculated to be $\approx 65\%$ (Fig S13).

[2 + 2]-Photodimerisation of **MTM-POEGA₄₈₀** was then performed using a mercury lamp with a bandpass filter (Omnicure s2000, 200 W, $\lambda = 320\text{--}390 \text{ nm}$). A kinetic investigation revealed that full conversion was reached after 9 mins, which is similar to that observed with **MTM-PEG** (Fig 4). Successful coupling was confirmed by SEC which showed the expected shift in the molecular weight

distribution following photocycloaddition ($M_n = 6400 \text{ g.mol}^{-1}$; $\mathcal{D} = 1.67$, Fig 3D). The asymmetry of the molecular weight distribution can be attributed to only $\approx 65\%$ of the chains containing the MTM-functionalised end group.

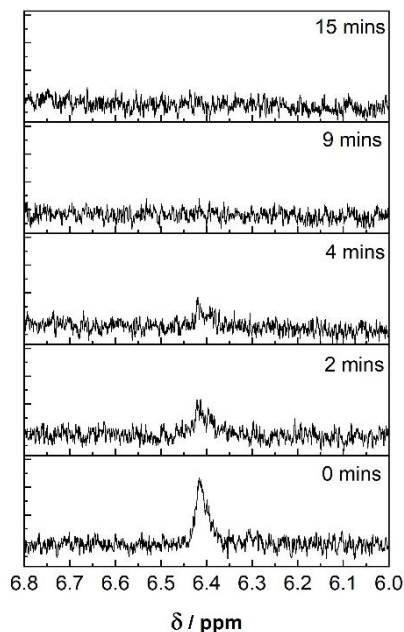


Fig 4. Kinetics of the [2 + 2]-photodimerisation of **MTM-POEGA₄₈₀** (200 W, $\lambda = 320\text{--}390 \text{ nm}$) determined by ^1H NMR following the signal of the olefinic proton at 6.40 ppm.

In summary, the [2 + 2]-photocycloaddition of monothiomaleimides has been successfully adapted to achieve [2 + 2]-photodimerisation of monothiomaleimide-functionalised polymers for the first time. Dimerisation of a linear PEG analogue (**MTM-PEG**) occurred, albeit slowly, in the presence of natural light and was greatly accelerated under UV irradiation. Kinetic investigation revealed that in a mixture of water/acetonitrile (95 : 5), the [2 + 2]-photodimerisation reached full conversion within 10 minutes upon exposure to UV light (Mercury lamp, 200 W, $\lambda = 320\text{--}390 \text{ nm}$ filter) resulting in dimerisation of the PEG chains. Under similar reaction conditions, a brush polymer synthesised by Cu(0)-mediated RDRP (**MTM-POEGA₄₈₀**) also underwent [2 + 2]-photocyclodimerisation, reaching full conversion within 9 minutes. The efficiency of the [2 + 2]-photocyclodimerisation reaction of these polymeric reagents and the ability to tune the absorbance spectrum of thiol-substituted maleimide derivatives alludes to potential broad application of this chemistry in synthetic polymer chemistry e.g. for compositional and architectural control and in materials science e.g. for precise spatiotemporal control over cross-linking reactions.

Conflicts of interest

There are no conflicts to declare.

Notes and references

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